21S103 Performance of the PRO-C3 collagen neo-epitope biomarker in non-alcoholic fatty liver disease

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INTRODUCTION & AIMS

There is an unmet need for non-invasive biomarkers in non-alcoholic fatty liver disease (NAFLD) that can diagnose advanced disease and identify patients suitable for clinical trials. The PRO-C3 collagen neo-epitope is a putative direct marker of fibrogenesis. **(Figure 1)**

We assessed the performance of PRO-C3 (within BEST diagnostic context of use) in a large, well-characterised international NAFLD cohort and report the development and validation of 2 novel panels for the diagnosis of advanced fibrosis (F≥3) in NAFLD, including a simplified clinical score which eliminates the need for online calculators.

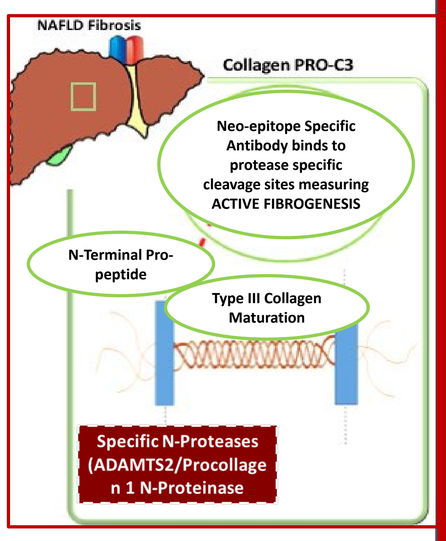

Figure 1: Overview PRO-C3 as an active marker of fibrogenesis

METHODS

Study Cohort: Plasma PRO-C3 levels were determined in a prospectively recruited international cohort of 449 patients with biopsy diagnosed NAFLD across the full disease spectrum (F0: n = 90; F1: 100; F2: 92; F3: 101; F4: 66). **Histology:** To reduce the element of inter-observer variability, over half of all biopsies (254, 57%) in our study were centrally reviewed by an expert member of the EPoS Histopathology Group (DT). A weighted kappa co- efficient of 0.90 for fibrosis stage was established, demonstrating a high level of inter- observer agreement. **PRO-C3 Levels:** Pro-C3 was assessed in EDTA plasma using a competitive enzyme-linked immunosorbent assay (ELISA, Nordic Bioscience A/S, Denmark), binding to N-Protease Cleavage site of Pro-collagen. Pro-C3 was centrally processed blinded and data then returned to independent (Newcastle) centre for analysis. **(Figure 2)**

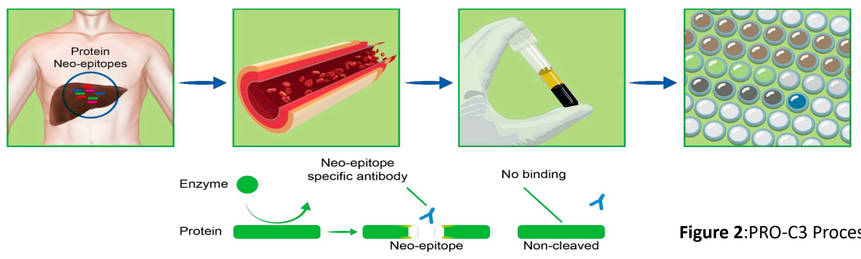

Figure 2:PRO-C3 Processing

Statistical Analysis: The cohort was divided into a discovery group (n = 151) and a validation group (n = 298). Logistic regression was performed to establish complex (FIBC3) and simplified (ABC3D) diagnostic scores to identify advanced fibrosis. The diagnostic accuracies of the models were determined by calculating the area under the receiver operating characteristic (ROC) curve. The Obuchowski measure was calculated to overcome the spectrum effect and ordinal scale issues associated with the 5-point fibrosis scale. The DeLong, DeLong and Clarke-Pearson method was used to compare AUROCs. Model cut-off point was selected using the Youden Index (J-Index).

RESULTS

PRO-C3 AS A SINGLE DIAGNOSTIC BIOMARKER

PROC3 levels correlated with steatohepatitis and fibrosis stage

PROC3 correlated with NAS score (Rs=0.304, p<0.0001) and fibrosis stage (Rs=0.422, p<0.0001). PROC3 exhibited the strongest correlation with fibrosis stage when compared to a number of other putative ECM turnover biomarkers (PROC6 (Rs=0.355), PROC4 (Rs= 0.279) and C4M (Rs=0.177), p<0.05).

DEVELOPMENT OF PANELS INCORPORATING PROC3 THAT ARE DIAGNOSTIC FOR ADVANCED FIBROSIS

The diagnostic panel "FIBC3" was calculated from the regression formula for prediction of severity of fibrosis: -5.939 + (0.053*Age) + (0.076*BMI) + (1.614*T2DM) – (0.009*platelets) + (0.071*PROC3).

The derived "ABC3D" score comprises: **A** = **A**ge>50 years, **B** = **B**MI>30, **C** = platelet **C**ount<200, **3** = PROC**3**>15.5ng/ml, **D**iabetes = present.

FIBC3 exhibited substantially improved accuracy (AUROC 0.89 and 0.83) in the discovery and validation sets, respectively) and outperformed FIB4 and other similar diagnostic panels. The simplified version, ABC3D, was concurrently developed and had comparable diagnostic accuracy (AUROC 0.88 and 0.81 in the discovery and validation sets, respectively). **Table 1.**

An optimal FIBC3 threshold value of >-0.4 was chosen using the Youden index (sensitivity 83%, specificity 80%, PPV 74% and NPV 88%). An optimal ABC3D cut-off level for the detection of advanced fibrosis was >3. (sensitivity 77%, specificity 82%, and accuracy 80%.).

Table1 Diagnostic Accuracy of Non-invasive Tests for Detecting Histologic Stage F3–F4 and Weighted AUROC Derived from the Obuchowski Measure

	Combined Cohort (n=449)		Discovery Cohort (n=151)		Validation Cohort (n=298)	
Non-invasive test	AUROC	Adj AUROC	AUROC	Adj AUROC	AUROC	Adj AUROC
AAR	0.67	0.62	0.66	0.62	0.66	0.62
APRI	0.75	0.68	0.75	0.69	0.75	0.68
BARD	0.71	0.67	0.76	0.69	0.69	0.66
FIB4	0.78	0.70	0.80	0.70	0.76	0.70
NFS	0.79	0.72	0.85	0.71	0.76	0.73
ADAPT	0.85	0.77	0.86	0.74	0.85	0.78
PROC3	0.76	0.69	0.75	0.68	0.78	0.70
FIB-C3	0.85	0.77	0.89	0.75	0.83	0.79
ABC3D	0.83	0.76	0.88	0.75	0.81	0.76
P-Value	<0.0001		<0.0001		<0.0001	

*Prevalence advanced fibrosis *combined cohort = 0.37 *Discovery cohort = 0.40 * Validation cohort = 0.36*DeLong DeLong Clarke test for comparison of AUROC

FIBC3 AND ABC3D PERFORMANCE SUPERIOR TO SIMPLE NON-INVASIVE SCORES

Performance characteristics of FIBC3 and the simplified ABC3D score were comparable to the recently described ADAPT score. FIBC3 and ABC3D have similar performance characteristics (p = 0.1422) as do FIBC3 and ADAPT (p=0.1859).

- Using the FIBC3 model, the optimal threshold correctly staged 224 out of 298 patients (75%) in the validation cohort, compared to 227 patients (76%) with ADAPT and 217 (73%) with ABC3D.
- Considering NPV, of 191 patients with mild fibrosis, 144 (75%) were staged correctly using FIBC3 or ABC3D, equal to ADAPT (75%).
- In the combined cohort (n=449), 347 of the patients (77%) were correctly staged using FIBC3, which out- performed both FIB4 at 304 (68%) and ADAPT at 341 (76%).

PERFORMANCE OF PROC3, FIBC3 AND ABC3D AS PRE-SCREENING TOOLS PRIOR TO LIVER BIOPSY TO SUPPORT CLINICAL TRIAL RECRUITMENT

The population modelled was "tdNASH", defined as NAS ≥4 with at least 1 point each for steatosis, hepatocyte ballooning and hepatic inflammation and fibrosis stage ≥F2.

PROC3 level >14.5ng/ml had an AUROC of 0.68 (sensitivity 59%, specificity 69%, accuracy 64%) for "tdNASH" detection. In general, tests incorporating PROC3 performed well. The most accurate test for the detection of tdNASH was FIBC3>-0.4 (71%). The availability of data from the currently recruiting Phase 2/3 clinical trials will be informative to further validate these findings

PROC3 MODEL INCORPORATING GENETIC INFORMATION (PNPLA3)

PROC3PNPLA3 model correctly staged advanced fibrosis in 71% of cases performing inferiorly to all PROC3 based models; FIBC3 (79%), ABC3D (74%) and ADAPT (77%).

Comparison of AUROC for the detection of advanced fibrosis showed no significant differences; PROC3PNPLA3 versus ABC3D (p= 0.0604); PROC3PNPLA3 versus FIBC3 (p= 0.2140) and PROC3PNPLA3 versus ADAPT (p= 0.1631), meaning that the addition of genetic information to a fibrosis detection model involving PROC3 did not improve the diagnostic accuracy therefore its inclusion cannot be recommended.

CONCLUSION

The current study provides a truly independent analysis of PROC3 biomarker performance for the diagnostic context of use **(Figure 3)**. The FIBC3 panel is an accurate tool with a single threshold value that maintains both sensitivity and specificity for the identification of F≥3 fibrosis, eliminating indeterminate results and outperforming commonly used non-invasive tools. A greatly simplified version (ABC3D) has been validated and shown to perform with similar accuracy, and may prove a useful tool in routine clinical practice.

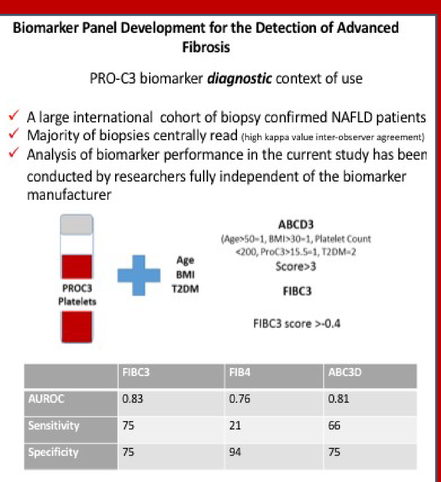

Figure 3:Study synopsis